

Examining the Temperature Dependence of Louche Formation in Absinthe

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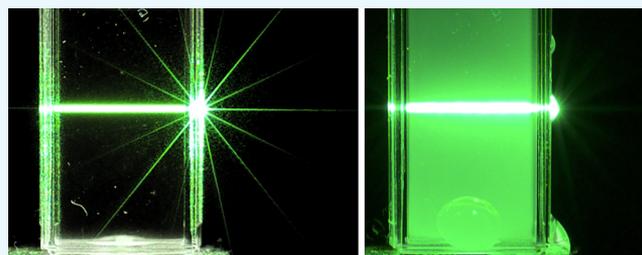
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ABSTRACT: Absinthe is an anise-flavored alcohol that is typically served by adding cold water to form a cloudy green louche, similar to the cloudy white louche of ouzo. This microemulsion formation, due to the competing interactions within the oil–alcohol–water system, has been termed the ouzo effect. Previous work has examined the ternary oil–alcohol–water phase diagram in ouzo and limoncello. Additional work has also characterized the droplet size and stability of microemulsions in ouzo, limoncello, and pastis. However, less work has been done to examine the effect of temperature on louche formation despite the fact that the louche is traditionally formed by adding ice cold water. This work demonstrates that both the maximum turbidity and the fraction of alcohol at maximum turbidity are temperature-dependent. The louche formation can be fit with a logistic curve, and the resulting fit parameters are linear with temperature. Optical images show that the increased turbidity correlates with an increase in the number of droplets in the microemulsion.



INTRODUCTION

Absinthe is an anise-flavored high-proof distilled alcoholic beverage, classified as a brandy that has been macerated with herbs. Historically, this green-colored beverage has been associated with hallucinations and madness, which led to it being banned in most countries by 1915¹ with the bans lasting until 1988 in the European Union² and 2007 in the United States.¹ It was thought that high quantities of thujone contained in wormwood oil that gives absinthe its distinct flavor² was the culprit behind these hallucinations. However, recent studies have demonstrated that the quantity of thujone (and of other possible hallucinogenic culprits such as antimony) are not high enough to cause the hallucinations, convulsions, and seizures of “absintheism,” and thus, the potency of the drink is due only to the high alcohol content,^{3,4} which can be as high as 74% (148 proof).

Absinthe is part of a family of alcohols flavored with essential oils that includes pastis and ouzo. These alcohols are commonly drunk by adding *cold* water to the drink, which turns a milky color due to the formation of an opalescent louche, as can be seen in Figure 1. It is interesting to note that Bohemian-style absinthe, a recipe that only contains wormwood and lacks all other essential oils, does not louche. Some research^{5–8} has been done to understand the physics of the louche formed in ouzo, pastis, and limoncello, because these alcohols were never banned as absinthe was. Results of that body of work indicate that the opalescent louche formed from each of these drinks is a microemulsion formed by the oil–water–alcohol interaction, with one phase (the dispersed

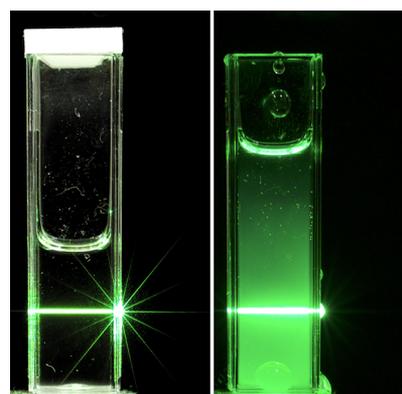


Figure 1. Images of an illuminated cuvette showing undiluted absinthe (left) and louché absinthe (right).

phase) consisting of oil–alcohol microdroplets contained within a second phase (the continuous phase) consisting of water dissolved in alcohol and containing the remaining fraction of the essential oils.⁸ At high enough water content, all of the

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essential oils are removed from the continuous phase.⁸ This spontaneous microemulsion formation in such alcohol–oil–water systems has been termed the ouzo effect.

Studies of this ouzo effect have examined the spinodal and binodal lines in the phase diagram. This has been done by making oil–alcohol–water solutions using a model oil so that a broad range of the ternary phase diagram can be explored.^{5,8} These papers have shown that there is both a region in the phase diagram with the ouzo effect and a region with a complete phase separation depending on the specific location of the spinodal and binodal boundaries. These studies have also shown that there are different droplet sizes in the emulsions depending on the specific oils with ouzo forming droplets of 1–2 μm ,⁵ while limoncello forms smaller droplets of ≈ 100 nm.⁸ This has implications both for taste, which is impacted by droplet size, and also in the stability of such microemulsions. Limoncello emulsions are very stable for a long period of time due to the small droplet size,⁸ whereas the other louches with larger μm droplets are less stable with time and the droplets sediment within ≈ 10 min for the actual alcohol although the model system is stable for a longer time.⁷ Most recently, research in the ouzo effect has looked at evaporating drops⁹ and demonstrated that this effect can occur in small droplets due to water evaporation at the surface. Other work looks to use the ouzo effect in order to form nanoparticles.¹⁰ However, little work has examined either absinthe itself or the effect of temperature on the formation of the emulsion. As the tradition is to utilize *ice cold* water to form the emulsion, this work examines the effect of temperature on the formation of a louche in absinthe.

RESULTS AND DISCUSSION

Figure 2 shows the optical transmission of the absinthe–water mixture (oil–alcohol–water) as a function of volume fraction

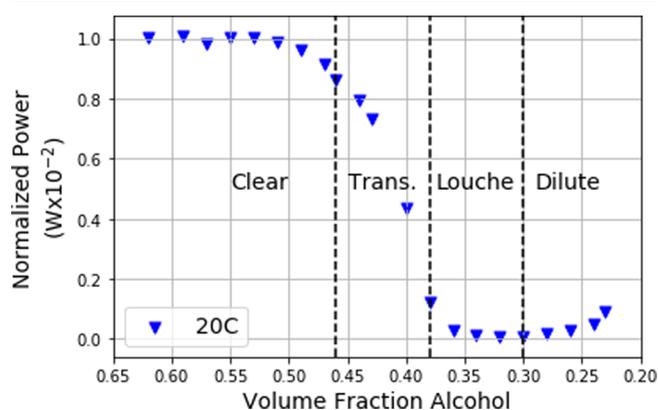


Figure 2. Dilution of absinthe at 20 °C. The x -axis is flipped so that a dilution runs from left (high EtOH) to right (low EtOH). The transition is defined as beginning when the transmitted power has decreased by 13.5% and completed when the power drops below 25%. The dilution region is defined as when the transmitted power begins increasing after a minimum value.

of ethyl alcohol (Ethanol, EtOH) taken at 20 °C. For this curve and also for the curves shown in Figure 3, water that was thermally equilibrated to the measurement temperature was pipetted in the cuvette and the solution was mixed thoroughly. The transmitted laser power was averaged for 120 s at 15 Hz at each dilution point. The transmitted optical power is a measure of solution turbidity: as the emulsion forms, dispersed

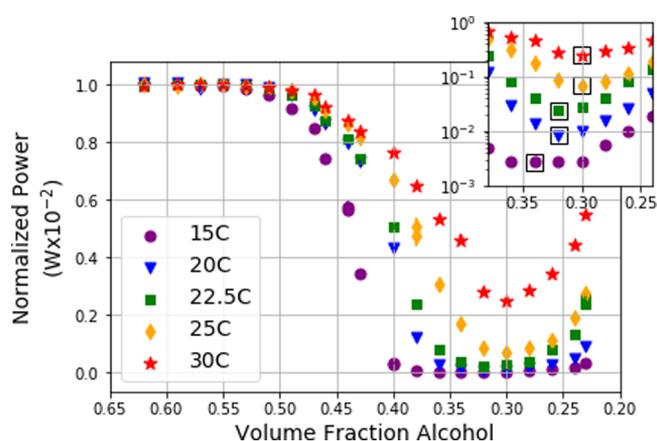


Figure 3. Dilution of absinthe at temperatures from 15 to 30 °C.

droplets scatter light out of the optical path, reducing the optical transmission. The transmitted power of the laser has been averaged over the first four data points and then normalized to that maximum value. During dilution, EtOH volume fraction decreases. In order to make the graph more intuitive to read, we have flipped the x -axis to go from high EtOH on the left to low EtOH on the right so that the dilution runs from left to right.

The 20 °C curve shown in Figure 2 shows the common shape and features of all the temperatures we measured. The optical transmission transitions smoothly from maximum (1) on the left to ≈ 0 on the right as water is added. This decrease in transmission corresponds to the formation of a louche. The optical transmission varies smoothly, with no discontinuous jumps, during the dilution. The resulting curve can be best described by dividing it into four different sections. Initially, the solution is clear and the entire laser beam power is transmitted, as shown in Figure 1 (left). We define the transition to begin when the power has dropped by 13.5% ($= e^{-2}$) (which also corresponds to when the solution can be identified by eye as not clear). In the transition region, the turbidity of the solution slowly increases (transmission decreases) until the entire solution appears cloudy at the completion of the transition region; see Figure 1 (right). We consider the solution as louched when the transmitted power drops below 25% (the transmitted power of the weakest louche we measured and also a point at which the solution appears louched by eye). Continuing to add water after the optical transmission reaches a minimum value results in the transmitted optical power increasing. Taken as a whole, and examining the formation of the louche in limoncello,⁸ our transmission data suggests that as water is added, a microemulsion is formed consisting of a dispersed oil-rich droplet phase within a EtOH–water continuous phase. As additional water is added, the turbidity increases as the oil-rich phase precipitates forming microdrops. After all the oil-rich phase has precipitated, continued addition of water only serves to dilute the dispersed phase, resulting in increased optical transmission.

It should be noted that homogenization when adding a volume of water is essential. When we did not homogenize, we saw spikes and dips in the transmission that could be attributed to whisps of microemulsion, resembling localized cirrus clouds that drifted in and out of the laser path. This resulted in a significant amount of noise in the data and lack of reproducibility, which was eliminated by homogenization.

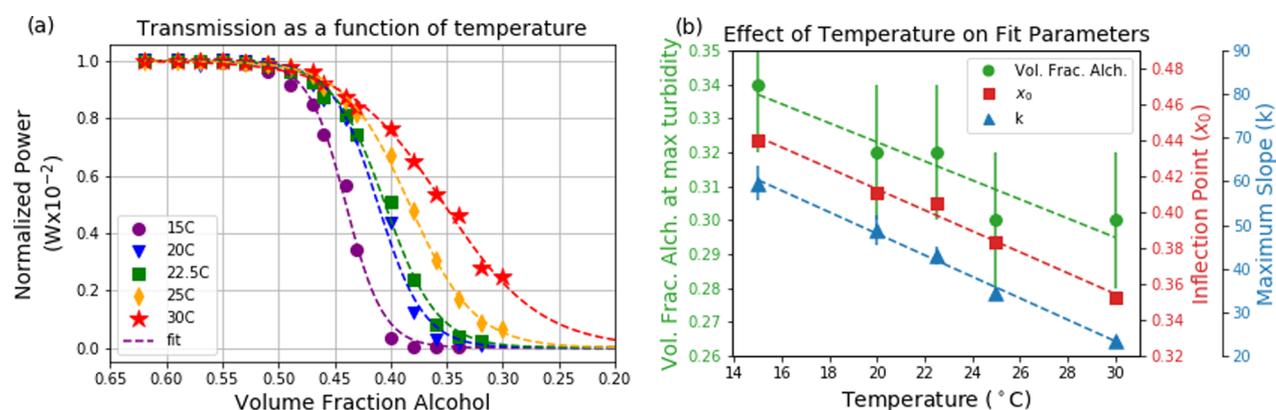


Figure 4. (a) Plot of normalized transmitted power vs volume fraction of alcohol for points up to the maximum louche and fitted with a logistic curve. (b) Plots of the parameters in the logistic curve vs temperature.

In order to examine temperature effects on the louche formation in absinthe, we examined five temperatures ranging from 30 °C (red star) to 15 °C (purple circle) and the dilution curves can be seen in Figure 3. In all cases, the laser transmission began at 7×10^{-2} W. This was normalized to 1 by averaging over the first four measurement points. Measurements were not taken below 15 °C, because at lower temperatures, condensation on the outside of the jacked beaker interfered with laser transmission and prevented optical measurements. As observed for the 20 °C dilution, the change in solution turbidity during the dilution is smooth at all temperatures, and the optical transmission is nearly constant until approximately 0.5 EtOH volume fraction. After this point, the transmission decreases smoothly until a minimum at 0.30–0.34 volume fraction of alcohol is reached and the turbidity of the solution is maximized. However, the temperature of the solution clearly impacts the louche in (1) the maximum turbidity of the louche, (2) the fraction alcohol at the minimum transmission point, and (3) the width of the transition region.

As shown in Figure 3, the maximum turbidity is clearly impacted by temperature. The transmitted laser power for the 30 °C sample is 25% and decreases to 7% at 25 °C and to 0.3% at 15 °C. Thus, the louche is more turbid at lower temperatures. We also see that the location (volume fraction alcohol) at the minimum transmission shifts with temperature. This is most easily seen in the inset of Figure 3, which plots the data on a log scale. The minimum for each curve is outlined in a square, and we can see from this that not only does the minimum power transmitted decrease with decreasing temperature but also the volume fraction of EtOH at that minimum increases with decreasing temperature from 30% at 30 °C to 34% at 15 °C. Finally, the width of the transition region increases with increasing temperature. This is perhaps most easily seen in the slope of the curve as it goes through the transition with a steeper slope for the 15 °C and a shallower slope for the 30 °C transition. We can quantify this by curve-fitting the louching process.

This data suggests that there are significant thermal effects on microemulsion formation in absinthe. In order to quantify the louching process better, we examined the points of the curve in the clear/transition/louche regions (ignoring data points in the dilution region). These points exhibit the s-shape of a logistic function. (Note: the s-shape appears backward because the x-axis of the graph was flipped so the dilution

proceeds from left to right.) The data were fit using a 2-parameter logistic function of the form

$$f(x) = \frac{1}{1 + e^{-k(x-x_0)}}$$

The numerator is 1 because the maximum power, as determined by the average of the first four data points, was normalized to 1. The curve has two fit parameters, x_0 and k , which are the inflection point of the curve and maximum slope of the curve, respectively. The R^2 values of the fit, which describe the residuals or error of the fit, are between 0.994 and 0.999 for each of the curves, and the shape of the fits, which are the dotted lines in Figure 4a, agree well with the data. The determined values of the fit parameters, x_0 and k for each temperature, are shown in Figure 4b as the red square and blue triangle points, respectively. The error bars on these points are equal to the error of each of the fits. The volume fraction EtOH for maximum turbidity is also plotted as the green circles, with error bars of 0.02, which is equal to the dilution step size. All of these fit parameters appear to have a linear dependence with temperature as can be seen by the dotted lines that are OLS linear fits. The R^2 value of the fit for the volume fraction of alcohol is slightly low at $R^2 = 0.875$, which is to be expected given the impact of the dilution step size (and large error bars). However, the linear fit to the logistic fit parameters are excellent with R^2 values of $R_{x_0}^2 = 0.987$ and $R_k^2 = 0.995$, suggesting that there is a clear linear dependence with temperature for these parameters.

A microemulsion is a spontaneously formed dispersion of drops within a continuous phase. Fitting logistic functions to the transmission data could correspond to a change in the number of drops in the microemulsion, the size of drops in the microemulsion, or both. We utilized optical microscopy to examine the size and number density of droplets in the microemulsion. Optical microscopy images, shown in Figure 5, were taken at two different temperatures and three different dilutions. The top row shows a dilution from left (40% EtOH) to right (26% EtOH) at 22.5 °C and the bottom row is at an elevated temperature of 33 °C. (Note: no image is included for 40% EtOH at 33 °C because no droplets were seen in this sample.) As can be seen from the images, the droplet size is approximately constant in all of the images with a diameter $1 \pm 0.1 \mu\text{m}$ when we examine the droplets that are in the focal plane for each image. Due to the constant Brownian motion of the droplets and the low number of droplets at some dilutions,

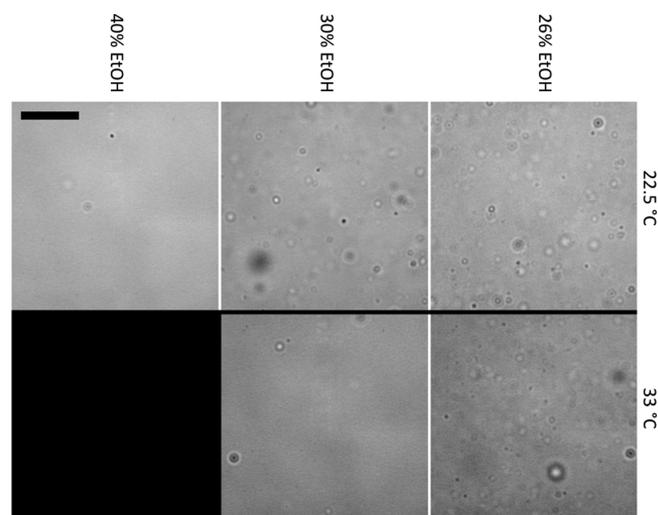


Figure 5. Brightfield optical microscopy images obtained at two different temperatures (22.5 and 33 °C) and three different dilutions (26, 30, and 40% EtOH). Scale bar = 10 μm . Fluids were thermally equilibrated prior to mixing. The droplet size is approximately monodisperse and constant diameter = 1 μm , while the droplet density strongly decreases with both increasing temperature and increasing EtOH concentration. Note: no oil-rich droplets were observed at 40% EtOH and 33 °C.

it is difficult to get a statistically significant number of droplets in focus in a single image, but the droplet size of 1 μm is in agreement with the sizes measured for ouzo.⁵ There may be some small change in size with temperature as was seen in ouzo, but that is beyond the resolution of this setup to image optically. However, the droplet density strongly varies both with temperature and EtOH concentration. There is an increase of droplet number and density with decreasing alcohol concentration and also with decreasing temperature. Both of these correspond with an increase in turbidity as measured in transmission, suggesting that the increase in turbidity corresponds to a higher number of droplets in the louche.

Finally, it is worth noting that the above analysis with turbidity assumes that there are not wavelength-specific impacts, such as changes in the droplet size, impacting our measurements. While the above optical microscopy images

show that there are not *large* size changes, it is unable to resolve small changes in droplet size. Ouzo is known to have small changes in size with temperature from 1.99 to 1.21 μm when increasing the temperature from 25 to 50 °C. Thus, in order to confirm that the data taken with the laser is descriptive of the system, we utilized UV–vis spectroscopy of the sample at room temperature. The results of both the transmission and the scattering at 90° is shown in Figure 6. Both curves have been normalized to 100% using a baseline scan of the halogen source with an empty cuvette. We can see in the transmission graph that pure absinthe has two characteristic absorptions at 414 and 650 nm. As louching occurs within the sample, the amount of light transmitted at all wavelengths decreases. This corresponds with an increase in scattering, as can be seen in the adjacent graph which has a broad peak between 450 and 850 nm with a maximum at 580 nm. Together, this data suggests that the 532 nm wavelength laser used above to characterize the sample is a good choice because it is far away from the characteristic peaks of the absinthe and near the position with the largest decrease in transmission.

CONCLUSIONS

This paper examines the effect of temperature on the formation of a microemulsion through the ouzo effect by examining the louching (turbidity) of absinthe diluted with water at different temperatures. We see a clear effect due to temperature. Warmer temperature mixtures form a less turbid louche and require more dilution (a lower volume fraction alcohol), while cold water dilutions form a more turbid louche with less water. Further, this transition can be fit with a logistic curve, and the fit parameters of inflection point and maximum slope follow a linear relationship. Finally, we show that this increase in turbidity corresponds to an increase in the number of droplets forming the microemulsion. Ultimately, this understanding of the temperature effect on the emulsion formation in these oil–alcohol–water mixtures can help us exploit the ouzo effect to form nanoparticles.

MATERIALS AND METHODS

The absinthe chosen was Lucid Absinthe, from The Combiar Distillery, Saumur, France. Lucid is one of the traditional absinthes distilled from Grande Wormwood (*Artemisia*

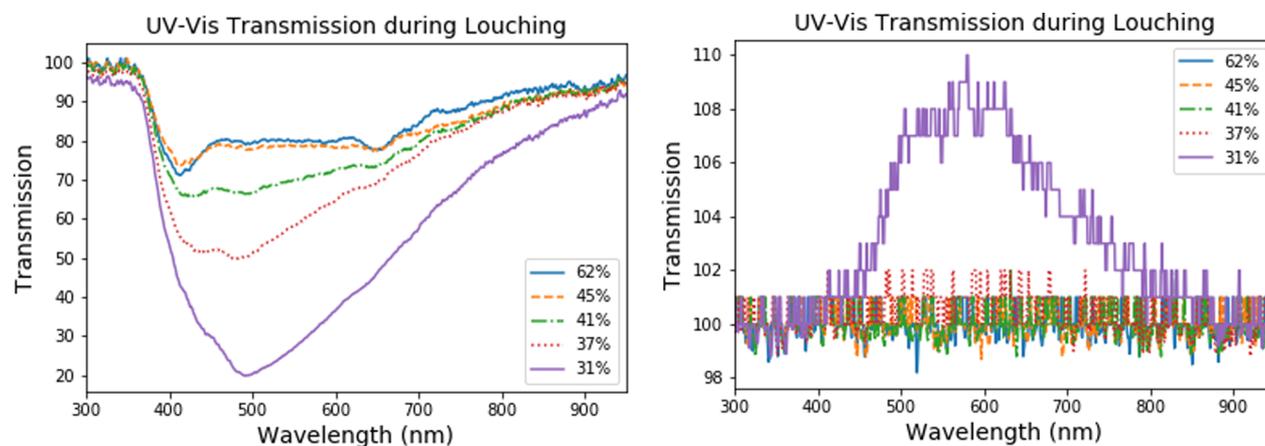


Figure 6. UV–vis spectroscopy of louching in transmission (a) and 90° scattering (b). Both curves have been normalized to 100% using a baseline scan of the halogen source with an empty cuvette.

absinthium). The recipe is proprietary and also includes green anise and sweet fennel to create a solution that is 62% alcohol by volume (124 proof), with the remainder being water, wormwood oil, and other oils and flavorings. While we do know the concentration of ethanol (from the proof), and by extension the approximate amount of water (as the oil is a very small volume), we do not know exactly what essential oils are present in our experimental sample or in what quantity. We can only provide an estimate of the oil fraction, which we determined to be less than 0.1% volume fraction based on centrifugation of the louché absinthe. As the exact oil composition is unknown, we are not able to independently alter the amount of essential oil in the sample. This means we can only alter the relative concentration of oil by adding water or pure ethanol. Many studies of alcohol louchés have used model systems with a single essential oil, water, and alcohol such as the lemon essential oil used by Chiappisi and Grillo.⁸ That will not work in this case for two reasons. First, commercially available wormwood oil has an unspecified amount of water and alcohol or other unspecified additional ingredients. But more importantly, it has previously been shown that Bohemian-style absinthe with only wormwood oil with little to no anise does not louché. For this reason, we have used an actual absinthe rather than a model system. However, it was demonstrated by Chiappisi and Grillo⁸ that the louché formed by actual lemoncello and that formed by a model system with lemon oil, water, and alcohol, is essentially the same, suggesting that additional oils herbs and flavors will have an effect on the fine details but not on the overall trends seen in the formation of the microemulsion.

In this paper, we diluted the absinthe with water. The added water was generated by reverse osmosis (Milli-Q) and was specified in terms of the electrical resistance (18.6 MOhm/cm). All fluids were dispensed using calibrated Gilson Pipetman and ThermoScientific pipettes.

We probed the phase transition optically and macroscopically, by measuring the unscattered transmission of laser light through a sample. Prior to louching, the absinthe–water mixture is a transparent continuous phase that transmits the majority of the laser light. The louché microemulsion is highly turbid, scattering the majority of the light. Each sample was illuminated by a 10 mW green CW laser (Crystalaser CL532-010-L) with stated power output stability as 0.5% over 24 h. The laser light was chopped before it reached the sample by a LaserProbe CTX-515 chopper, powered by a Electro Industries model 3002A regulated DC power supply to provide improved signal-to-noise measurements. The sample itself was held by a 50 mL jacketed reaction beaker (Kontes, now part of Kimble Chase Life Science and Research Products, LLC) used to maintain and control sample temperature. The transmitted laser light was detected by an RSP-590 radiometer head, and the entrance pupil of the pyroelectric head was small enough to reject scattered light. The measured power level was then digitally sampled using a NI data acquisition module (part number 154424C-03L). An image of the temperature controlled beaker can be seen in Figure 7. Labels (a,d) highlight the outlet and inlet for the tubing that connects the beaker to the water chiller (removed for clarity). The jacketed beaker has a copper inset (c) that both holds the beaker in place so that it does not shift during the measurement and contains a notch to accept the brass base that holds the cuvette. The cuvette, (b), can be seen in the center of the beaker with some liquid inside it. A screw holds the cuvette securely in

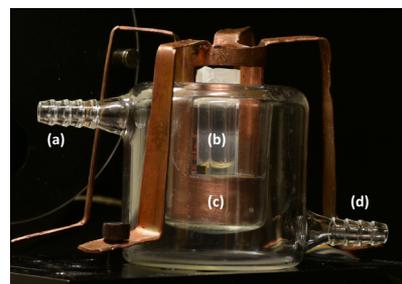


Figure 7. Sample holder. This photograph shows details of our sample holder, showing the jacketed beaker fluid line output (a), the partially-filled cuvette (b), the holder base (c), and jacketed beaker fluid line input (d).

place and allows careful alignment of the cuvette so that one face is normal to the incoming laser light. Once the cuvette is loaded within the beaker, water is added to fill the cavity. This maximizes thermal transmission from the jacketed beaker to the cuvette that occurs through both the brass holder at the base and the water bath on the sides of the cuvette. Thermal stability of the setup was checked with a K-type wire thermocouple probe read by a Digisense type J/K thermocouple meter placed within a water-filled cuvette. Measurements show that thermal stability within the jacketed beaker cavity was held within 0.5 °C of the water bath temperature, and an equilibration of ≈ 16 min at temperature was sufficient for the cavity to match the bath temperature.

Dilution measurements were taken as follows: The experimental setup was first allowed to equilibrate to a desired temperature for 30 min. The absinthe was pipetted into the cuvette and allowed to equilibrate for a further 10 min. Fluid to be added to the sample was pre-chilled (or pre-heated) within a small holding chamber in the water bath and added to the sample in increments using a micropipette. After adding the fluid, the sample was mixed by aspirating and dispensing several times with the micropipette. We found that mixing was essential to homogenize the sample; passive diffusion was insufficient. Intensity data for each point was acquired for 120 s at 15 Hz and averaged over this time. A point was re-run if there was drift during this time. Capping the cuvette prevented evaporation during experiments.

UV–vis spectroscopy was taken using a halogen light source and a StellarNet Black C-SR-50 spectrometer. A baseline scan was taken of the halogen source and empty cuvette, and all curves were normalized relative to this baseline. The recorded curves are averaged over 10 scans with a 100 ms integration time.

In parallel, images of louché samples were obtained at room temperature and at elevated temperature using a stage heater. Images were acquired using a 100 \times NA 1.47 microscope objective (Leica) and 30 fps imaging sensor (Flea, Point Grey Research) using standard brightfield illumination. Water and absinthe were held on the heated stage at the imaging temperature, combined to form the louché, and then imaged in closed sample holders on the heated stage to minimize evaporation.

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Notes

The authors declare no competing financial interest.

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